19082

a. Paragraph (d) is amended as follows:

i. By changing the word "should" to "shall" wherever it appears in paragraphs (d)(1) (ii) and (iii)(B), (3), and (8)(ii).

ii. By changing the word "should" to "shall" in certain sentences in paragraph (d)(2), which is revised to read as follows:

(2) *Number of animals.* At least eight animals of each sex should be used at each dose level and should be designated for behavioral testing. If interim sacrifices are planned, the number should be increased by the number of animals scheduled to be sacrificed before the end of the study. Animals shall be randomly assigned to treatment and control groups.

iii. By changing the word "should" to "shall" in certain sentences in paragraph (d)(8)(i), which is revised to read as follows:

(i) All animals in a given study should be observed carefully by trained technicians who are blind with respect to the animals' treatments. Standard procedures to minimize observer variability shall be followed. Where possible, it is advisable that the same observer be used to evaluate the animals in a given study. If this is not possible, some demonstration of inter-observer reliability is required. All animals should be observed prior to initiation of exposure. Subsequent observations should be made with sufficent frequency to ensure the detection of behavioral and/or neurologic abnormalities, if present. At minimum, observations at 1 hour, 6 hours, 24 hours, 7 days, and 14 days and monthly thereafter are recommended. In a subchronic study, subsequent to the first exposure all observations should be made before the daily exposure. The animals should be removed from the home cage to a standard arena for observation. Effort should be made to ensure that variations in the test conditions are minimal and are not systematically related to treatment. Among the variables that can affect behavior are sound level, temperature, humidity, lighting, odors, time of day, and environmental distractions. Explicit, operationally defined scales for each function should be used. The development of objective quantitative measures of the observational endpoints specified is encouraged.

b. By changing the word "should" to "shall" in certain sentences in paragraph (e)(3), which is revised to read as follows:

(3) *Evaluation of data.* The findings of a functional observational battery should be evaluated in the context of preceding and/or concurrent toxicity studies and any correlative histopathological findings. The evaluation shall include the relationship between the doses of the test substance and the presence or absence, incidence and severity, of any neurotoxic effects. The evaluation should include appropriate statistical analyses. Choice of analyses should consider tests appropriate to the experimental design and needed adjustments for multiple comparisons.

§ 798.6200 [Amended]

20. Section 798.6200 *Motor activity* is amended in paragraph (d)(1)(iii)(B) by changing the word "should" to "shall" wherever it appears.

§ 798.6400 [Amended]

21. Section 798.6400 *Neuropathology* is amended as follows:

a. Paragraph (d) is amended by changing the word "should" to "shall" wherever it appears in paragraphs (d)(1) (i) and (iii) and (8) (ii)(C), (iii), and (iv)(B).

b. Paragraph (e) is amended by changing the word "should" to "shall" wherever it appears in the introductory text and in paragraph (e)(1).

[FR Doc. 87-11124 Filed 5-19-87; 8:45 am]
BILLING CODE 6560-50-M

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40 CFR Parts 795 and 799

[OPTS-42033D; FRL 3202-3]

Cresols; Final Test Standards and Reporting Requirements

AGENCY: Environmental Protection Agency (EPA).

ACTION: Final rule. Phase II

SUMMARY: EPA is issuing a final rule under section 4(a) of the Toxic Substances Control Act (TSCA) that specifies test standards and reporting requirements for testing of ortho (o), meta (m) and para (p) cresols (CAS 95-48-7, 108-39-4, 107-44-5).

DATES: In accordance with 40 CFR 23.5 (50 FR 7271; February 21, 1985), this rule shall be promulgated for purposes of judicial review at 1 p.m. eastern ["daylight" or "standard" as appropriate] time on June 3, 1987. This rule shall become effective on July 6, 1987.

FOR FURTHER INFORMATION CONTACT: Edward A. Klein, Director, TSCA Assistance Office (TS-799), Office of Toxic Substances, Rm. E-543, 401 M St. SW., Washington, DC 20460, (202) 554-1404.

SUPPLEMENTARY INFORMATION: In the Federal Register of April 28, 1986 (51 FR 15771), EPA issued a final Phase I rule under section 4(a) of TSCA to require testing of cresols for mutagenic effects, developmental toxicity, and reproductive effects. Also, in that issue of the Federal Register, EPA issued a proposed Phase II rule which named the test guidelines to be used for testing and proposed that tests be submitted within specific time frames (51 FR 15803; April 28, 1986). The Agency is now promulgating a final Phase II rule specifying these test standards and reporting requirements for this testing. This test rule for cresols is being promulgated under 40 CFR 799.1250.

I. Background

The Phase I final rule specified the testing requirements for cresols: (1) Mutagenic effects studies (including tests for chromosomal aberrations, gene mutations, and cellular transformations) on specified cresol isomers; (2) a developmental toxicity study with each cresol isomer; and (3) a two-gengration reproductive effects study with each cresol isomer.

Sections 790.50 and 790.52 of Title 40 of the Code of Federal Regulations (CFR) discuss the test standard rule development procedure. In the case of the cresols test rule, which was initiated under the two-phase process, EPA decided to propose the relevant TSCA test guidelines as the test standards. In addition, EPA proposed that the data from the required studies be submitted within certain time periods. These time periods serve as the data submission deadlines required by TSCA section 4(b)(1). The reasons for this change in the test rule development process for cresols were discussed in the proposed rule.

II. Modifications to the Two-Phase Rulemaking Process

Because EPA proposed certain TSCA guidelines as the test standards and proposed data submission deadlines, persons subject to the Phase I final rule were not required to submit proposed study plans for the required testing or proposed dates for the initiation and completion of that testing. They were, however, still required to submit notices of intent to test or exemption applications in accordance with 40 CFR 790.45.

On July 8, 1986, the Cresols Program Panel (the Panel) of the Chemical Manufacturers Association (CMA) notified EPA of its intent to conduct the testing required in the Phase I test rule for cresols (Ref. 1). In addition, the

Procter and Gamble Co. and the Sigma-Aldrich Corp., processors of cresols, requested exemptions (Refs. 2 and 3, respectively). EPA is now requiring the test sponsor to conduct testing in accordance with specific test standards and reporting requirements. These standards and reporting requirements reflect the Agency's evaluation of comments received on the proposed rule. Moreover, once this Phase II final rule is promulgated, those persons who have notified EPA of their intent to test must submit study plans (which adhere to the promulgated test standards) no later than 45 days before the initiation of each of the required tests.

III. Proposed Phase II Test Rule

A. Test Standards

In the proposed Phase II rule, the Agency proposed that testing of cresols be conducted using the following TSCA test guidelines as test standards:

1. *Mutagenicity.* Chromosomal effects.

a. *In vitro* mammalian cytogenetics test (40 CFR 798.5375).

b. *In vivo* mammalian bone marrow cytogenetics tests: chromosomal analysis (40 CFR 798.5385).

c. Rodent dominant lethal assay (40 CFR 798.5450).

2. *Mutagenicity.* Unscheduled DNA synthesis in mammalian cells in culture assay (40 CFR 798.5550).

3. *Mutagenicity.* Gene mutations.

a. Detection of gene mutations in somatic cells in culture assay (40 CFR 798.5300).

b. Sex-linked recessive lethal test in *Drosophila melanogaster* (40 CFR 798.5275).

4. *Mutagenicity.* Cellular transformations. Morphologic transformation of mammalian cells in culture assay (40 CFR 795.285).

5. *Developmental toxicity.* Developmental toxicity study (40 CFR 798.4900).

6. *Reproductive effects.* Reproduction and fertility effects study (40 CFR 798.4700).

EPA also proposed that the revisions to these guidelines, which were proposed in the **Federal Register** of January 14, 1986 (51 FR 1522), be adopted in the test standards for cresols. EPA has responded to comments concerning these guideline revisions in the record for rulemaking (Ref. 5). These guidelines are discussed and promulgated elsewhere in this issue of the **Federal Register** and are included in this rulemaking docket. In addition, EPA proposed several chemical-specific test standard modifications such as route of administration of test substances, multiple dosing, specific strains, cell lines and species, solvents, negative controls, and specific activation systems. For additional information on proposed test standards and supporting rationale for modifications, consult the proposed Phase II rule on cresols (51 FR 15803; April 28, 1986).

B. Reporting Requirements

EPA proposed that all data developed under this rule be reported in accordance with its TSCA Good Laboratory Practice (GLP) standards (40 CFR Part 792). In addition, test sponsors are required to submit individual study plans at least 45 days prior to beginning each study. The Agency has modified this requirement in a procedural rule published in the **Federal Register** of June 30, 1986 (51 FR 23706). The Agency also proposed specific reporting requirements for each of the proposed tests. For additional information on proposed reporting requirements, consult the proposed Phase II rule on cresols (51 FR 15803; April 28, 1986).

IV. Response to Public Comments

The Agency received comments from the CMA Cresols Program Panel (Ref. 4). The major issues identified during the comment period and EPA's responses to those comments are discussed below.

A. Reporting Requirements

The Panel recommended that the reporting schedules proposed in the Phase II rule be reevaluated. It recommended that all the 12-month reporting schedules be extended to 18 months and that the 29-month reporting schedule for the reproductive effects test be extended to 36 months. The Agency disagrees with the Panel's comments and believes that the proposed reporting schedules are realistic because the Agency's schedule already takes into account the longest possible sequential route of testing, considering administrative and logistical variables specific to each test. These time variables were then added cumulatively to the time required to perform the tests. The Agency did extend the reporting deadline for the *in vivo* mammalian bone marrow cytogenetics test from 12 months to 14 months. This will allow for the *in vivo* testing to follow the negative *in vitro* tests.

B. Repeating Mutagenicity Assays

The CMA Panel commented on the generic requirement to repeat all mutagenicity assays. In particular, the Panel commented on this requirement for the gene mutation in cells in culture assay, the cell transformation assay, and the sex-linked recessive lethal assay in *Drosophila*. The Panel argues that repetition of tests, in some cases, is redundant and would not yield more useful information.

The Agency agrees that a generic requirement to repeat all *in vivo* mutagenicity assays is not routinely necessary; however, the Agency believes that under certain conditions repeats of tests are appropriate and necessary. The Agency interprets any single positive finding at one dose level, but no dose response, as a positive mutagenic response in the absence of a repeat assay. The Agency is therefore not including a generic requirement for repeats of the following assays: *in vivo* mammalian cytogenetics, *Drosophila* sex-linked recessive lethal, and rodent dominant lethal. Because of the nature of *in vitro* tests in comparison to *in vivo* systems, the Agency believes that repeats of equivocal studies are appropriate and necessary for the evaluation of the *in vitro* mammalian cytogenetics, the gene mutation in somatic cells in culture, and the morphologic transformation of mammalian cells in culture assays. The Agency is thus requiring repeats of these *in vitro* assays over a narrow range of concentrations in the event a single, statistically significant increase is produced at one dose point without a dose response.

C. In Vivo Cytogenetics Assay

The Panel recommended that, because there are available LD_{50} data on cresols, the high dose of the three dose levels for this assay be one-tenth of the acute LD_{50} for the test material in the mouse by the oral route. The middle and low test dose should be logarithmically placed.

The Agency believes that there is not adequate reason to proceed as the Panel recommended. This highest dose tested must be the maximum tolerable dose or that producing some indication of cytotoxicity. There is no reason to believe that one-tenth of the available LD_{50} for cresols will be sufficient to cause some cytotoxicity. Therefore, the Agency does not agree with the Panel's suggested modification to the *in vivo* cytogenetics assay.

D. Detection of Gene Mutations in Somatic Cells in Culture

The Panel recommended that cresols be tested in Chinese hamster ovary (CHO) cells rather than the L5178Y mouse lymphoma cells proposed by the Agency for this assay. The Agency specifically proposed the L5178Y cells because of the previous assays with a cresols mixture and with *o*-cresol using that cell line. EPA is interested in obtaining the clearest overall picture of

the mutagenic effects of each of the cresol isomers. Therefore, for the two isomers, *m*-cresol and *p*-cresol, for which testing in this assay is required, the Agency disagrees with the Panel on the use of the CHO cells. The required testing should continue with the L5178Y cells which were used in previous gene mutation assays on cresols.

The Panel also recommended that as part of the test results criteria for this assay, a consideration of a dose dependent increase in mutation and an absolute increase in mutant colony number be included. The Panel stated that this increase should be statistically significant. The section of the TSCA test guideline for this assay that deals with the interpretation of results (40 CFR 798.5300) states that either of the above criteria could be used to determine positive test results.

E. Morphological Transformation of Mammalian Cells in Culture

The Panel recommended that the C3H10T½ mouse embryo cell is a more appropriate test system for this assay and that this cell line be used instead of the Balb/c–3T3 mouse cells which the Agency proposed. Because of a previous positive result in a cellular transformation assay with a mixture of the three cresol isomers, the Agency proposed the use of Balb/c–3T3 cells. The Agency has determined that because of the existing data using this cell line with cresols, the cell line for this assay should continue to be the Balb/c–3T3 cells.

The test criteria for the cell transformation assay are based on those criteria which were used for previous Balb/c–3T3 cell transformation assays on an equimixture of the three cresol isomers, for which a positive result was obtained. The Agency is interested in continuity of test performance so that the existing results can be compared with future test results.

In addition, the Panel does not agree with some of the test criteria included in the standard for determining a positive result. While the Panel did agree with the need for demonstration of a dose-response as a criterion for a positive evaluation, the Panel did not believe that the detection of a reproducible and statistically significant positive response in only one of the test substance concentrations supports a positive finding. Instead, the Panel recommended that a determination of positive evidence for induction of transformation be the demonstration of a dose-related increase in the number of transformed foci and a six- to- eightfold increase over identified background transformation rates. The Panel believes that if both conditions are met, the test substance should be considered positive for cell transformation. If neither condition is met, the test substance should be considered negative. If only one condition is met, the test substance is equivocal and should be repeated, perhaps utilizing more appropriate test concentrations.

The section of this guideline which addresses the interpretation of results states the two criteria suggested by the Panel. However, the Agency has determined that either a statistically significant concentration-related increase or a statistically significant and reproducible positive response at any one dose level will indicate a positive response in this test. As indicated in Unit IV. B., the Agency believes that repeats of equivocal studies are necessary for the evaluation of *in vitro* mutagenicity assays. An unusually elevated response in an *in vitro* assay at a single data point, in the absence of a dose response, warrants a repeat assay over a dose range designed to bracket the dose of interest. If the repeat assay shows a statistically significant positive response for at least one of the test substance concentrations then the results are interpreted as positive.

V. Final Phase II Test Rule

A. Test Standards

The mutagenicity, developmental toxicity, and reproductive effects test guidelines and chemical specific modifications proposed for cresols and finalized in this Federal Register shall be the test standards for the testing of cresols under 40 CFR 799.1250 (see Unit III. A. of this preamble). The Agency believes that the conduct of the required studies in accordance with these test standards is necessary to assure that the results are reliable and adequate.

B. Reporting Requirements

All data developed under this rule must be reported in accordance with the TSCA GLP Standards (40 CFR Part 792). In addition, test sponsors are required to submit individual study plans at least 45 days prior to the initiation of each study in accordance with 40 CFR Part 790. This is a change from the proposed rule which stated 30 days (see 51 FR 23706; June 30, 1986).

The Agency is required by TSCA section 4(b)(1)(C) to specify the time period during which persons subject to a test rule must submit test data. On the basis of the Agency's regulatory experience with the health effects tests required for cresols, as well as in response to certain public comments, EPA is adopting the reporting requirements in Table 1 of this preamble. Accordingly, results for the required tests must be reported as specified. After issuing the proposed rule for cresols, the Agency decided that interim reports for the testing required for substances under section 4 of TSCA should be submitted at 6-month intervals rather than at 3-month intervals. This reporting frequency will be sufficient to keep EPA informed of the current status of required testing and of any difficulties which the testing facility may encounter during testing. This change also lessens the reporting burden of test sponsors. Accordingly, the final reporting requirements for the testing required for cresols reflect a requirement for 6-month, rather than 3-month, interim reports.

TABLE 1.—REPORTING REQUIREMENTS FOR CRESOLS

Test	Reporting deadline for final report (months after the effective date of final phase II rule).	Number of interim (6-month) reports required
In vitro mammalian cytogenetics test	12	1
In vivo mammalian bone marrow cytogenetics test	14	1
Rodent dominant lethal assay	24	3
Unscheduled DNA synthesis in mammalian cells in culture assay	12	1
Gene mutation in cells in culture assay	12	1
Sex linked recessive lethal test in *Drosophila*	24	3
Morphologic transformation of mammalian cells in culture assay	12	1
Oral developmental toxicity	12	1
Reproduction and fertility effects	29	4

TSCA section 14(b) governs Agency disclosure of all test data submitted pursuant to section 4 of TSCA. Upon receipt of data required by this rule, the Agency will publish a notice of receipt in the Federal Register as required by section 4(d).

C. Conditional Exemptions Granted

The final rule for test rule development and exemption procedures (40 CFR 790.87) indicates that, when certain conditions are met, exemption applicants will be notified by certified mail or in the final Phase II test rule for a given substance that they have received conditional exemptions from test rule requirements. The exemptions granted are conditional because they will be given based on the assumption that the test sponsors will complete the required testing according to the test standards and reporting requirements established in the final Phase II test rule for the given substance. TSCA section 4(c)(4)(B) provides that if an exemption

is granted prospectively (that is, on the basis that one or more persons are developing test data, rather than on the basis of prior test data submissions), the Agency must terminate the exemption if the test sponsor has not complied with the test rule.

Since sponsors have indicated to EPA by letter of intent (Ref. 1), their agreement to sponsor all of the tests required for cresols in the final Phase I test rule for this substance (51 FR 15771; April 28, 1986) and EPA has established test standards and reporting requirements in this final Phase II rule for cresols, the Agency is hereby granting conditional exemptions to all exemption applicants (Refs. 2 and 3) for all of the testing.

D. Judicial Review

The promulgation date for the cresols Phase I final test rule was established as 1 p.m. eastern daylight time on May 12, 1986. To EPA's knowledge, no petitions for judicial review of that Phase I final rule. Any petition for judicial review of this Phase II final rule will be limited to a review of the test standards and reporting requirements for cresols established in this rule.

E. Other Provisions

Section 4 findings, required testing, test substance specifications, persons required to test, enforcement provisions, and the economic analysis are presented in the final Phase I rule for cresols (51 FR 15771).

VI. Rulemaking Record

EPA has established a record for this rulemaking [docket number (OPTS-42033D)]. This record includes basic information considered by the Agency in developing this final rule and appropriate **Federal Register** notices.

A. Supporting Documentation

The supporting documents for this final rulemaking consist of: The proposed and final **Phase I test rule** on cresols (48 FR 31812; July 11, 1983 and 51 FR 15771; April 28, 1986, respectively). the proposed **Phase II test standards** rule on cresols (51 FR 15803; April 28, 1986), and the notice of Revision of TSCA Test Guidelines published elsewhere in this issue of the **Federal Register**.

B. References

(1) CMA. Chemical Manufacturers Association. Letter of intent to conduct testing on behalf of Cresols Program Panel from Geraldine V. Cox, to TSCA Public Information Office, U.S. Environmental Protection Agency. July 8, 1986.

(2) The Procter and Gamble Company. Letter requesting exemption from the test rule for cresols from James T. O'Reilly to TSCA Public Information Office, U.S. Environmental Protection Agency. June 9, 1986.

(3) Sigma-Aldrich Corporation. Letter requesting exemption from the test rule for cresols from David R. Harvey to Document Control Office, U.S. Environmental Protection Agency. June 5, 1986.

(4) CMA. Chemical Manufacturers Association. Comments on the EPA Proposed Testing Standards on behalf of the Cresols Program Panel from Geraldine V. Cox to TSCA Public Information Office, U.S. Environmental Protection Agency. June 12, 1986.

(5) USEPA. "Response to Public Comments, Proposed Revision of TSCA Test Guidelines as published in 51 FR 1522 (January 14, 1986)". Test Rules Development Branch, Existing Chemicals Assessment Division, Office of Toxic Substances, Environmental Protection Agency, Washington, DC (January 1987).

The record (except for documents containing TSCA confidential business information) is open for inspection from 8 a.m. to 4 p.m., Monday through Friday except legal holidays, in Rm. G-004, Northeast Mall, 401 M St. SW., Washington, DC 20460.

VII. Other Regulatory Requirements

A. Executive Order 12291

Under Executive Order 12291, EPA must judge whether a regulation is "major" and therefore subject to the requirements of a Regulatory Impact Analysis. This test rule is not major because it does not meet any of the criteria set forth in section 1(b) of the Order. The economic analysis of the testing of cresols is discussed in the Phase I test rule (51 FR 15771; April 28, 1986).

This final Phase II test rule was submitted to the Office of Management and Budget (OMB) for review as required by Executive Order 12291. Any written comments received from OMB, together with any EPA response to these comments, are included in the public record for this rulemaking.

B. Regulatory Flexibility Act

Under the Regulatory Flexibility Act (15 U.S.C. 601 *et seq.*, Pub. L. 96-354, September 19, 1980), EPA is certifying that this test rule, if promulgated, will not have a significant impact on a substantial number of small businesses for the following reasons:

(1) There are no small manufacturers of this substance.

(2) Small processors are not expected to perform testing themselves, or participate in the organization of the testing effort.

(3) Small processors are unlikely to be affected by reimbursement requirements.

C. Paperwork Reduction Act

The Office of Management and Budget (OMB) has approved the information collection requirements contained in the final Phase II rule under the provisions of the Paperwork Reduction Act of 1980, 44 U.S.C. 3501 *et seq.*, and has assigned OMB control number 2070-0033. No public comments on these requirements were submitted to the Office of Information and Regulatory Affairs of OMB.

List of Subjects in 40 CFR Parts 795 and 799

Testing, Environmental protection, Hazardous substances, Chemicals, Recordkeeping and reporting requirements.

Dated: May 4, 1987.

John A. Moore,

Assistant Administrator for Pesticides and Toxic Substances.

Therefore, Chapter I of Title 40 CFR is amended as follows:

PART 795—[AMENDED]

1. In Part 795:

a. The authority citation continues to read as follows:

Authority: 15 U.S.C. 2603.

b. By adding new § 795.285 to Subpart D, to read as follows:

§ 795.285 Morphologic transformation of cells in culture.

(a) *Purpose. In vitro* assays for cellular transformation are semi-quantitative assays for the ability of chemical agents to morphologically alter (transform) cells in culture. Such transformation is associated with certain phenotypic changes such as loss of contact inhibition and the ability to form colonies in soft agar medium. The process by which these changes occur is assumed to be closely related to the process of *in vivo* carcinogenesis. Morphologically transformed cells appear as foci of dense, piled-up, altered cells on an underlying monolayer of normal cells. Three types of foci have been recognized. Type III foci appear to be most closely correlated with *in vivo* tumor formation. The ultimate criterion for morphologic transformation is the ability of the transformed cells to induce tumors when inoculated into appropriate hosts. Not all cells which

appear to be morphologically transformed are capable of tumor formation. In general, there is reasonably good correlation between *in vitro* transformation and *in vivo* oncogenesis, although the correlation varies depending on the system being studied. These systems are believed to be reasonably good predictors of *in vivo* activity, and positive results are viewed as potential indications of *in vivo* carcinogenesis.

(b) *Definitions.* (1) Morphologic transformation is the acquisition of certain phenotypic characteristics, most notably loss of contact inhibition and loss of anchorage dependence, which are often but not always associated with the ability to induce tumors in appropriate hosts.

(2) Type III foci of transformed cells are multilayered aggregations of densely staining cells with random orientation and criss-cross arrays at the periphery of the aggregate. They appear as dark stained areas on a light staining background monolayer which is one-cell thick.

(c) *Reference substances.* Not applicable.

(d) *Test method*—(1) *Principle.* (i) Three systems for detecting chemically induced morphologic transformation have been described. They are:

(A) Systems which employ cell lines (cells with an indefinite lifespan).

(B) Systems which employ cell strains (cells with a finite or limited lifespan).

(C) Systems which detect the interaction between chemicals and oncogenic viruses.

(ii) This study shall employ an established cell line for detection of morphologic transformaton.

(2) *Description.* Cells in culture are exposed to the test substance, both with and without metabolic activation, for a defined period of time. Cytotoxicity is determined by measuring the colony-forming ability and growth rate of the cultures after the treatment period. At the end of the treatment period, cultures are maintained in growth medium for a sufficient period of time to allow near-optimal expression of transformed foci.

(3) *Cells.* (i) Balb/c-3T3 mouse cells originally obtained from clone A-31 or its derivatives shall be used in the assay. Cells shall be checked for mycoplasm contamination prior to use in the assay and may be checked for karyotype.

(ii) Appropriate culture media and incubation conditions (culture vessels, CO_2 concentrations, temperature, and humidity) shall be used.

(4) *Metabolic activation.* Cells shall be exposed to test substance both in the presence and absence of a metabolic activation system. The metabolic activation system shall be derived from primary cultures of rat hepatocytes.

(5) *Control groups.* Positive and negative (untreated and vehicle) controls shall be included in each experiment. 3-Methylcholanthrene is an example of a positive control for experiments without metabolic activation. Dimethylnitrosamine is an example of a positive control in experiments with metabolic activation.

(6) *Test chemicals*—(i) *Vehicle.* Test agents shall be dissolved in serum-complete culture medium prior to treatment of the cells.

(ii) *Exposure concentrations.* Several concentrations (usually at least four) of the test substance shall be used. These shall be selected on the basis of a preliminary cytotoxicity assay performed both with and without metabolic activation. The highest concentration shall produce a low level of survival (approximately 10 to 20 percent), and the survival in the lowest concentration shall approximate that of the negative control.

(e) *Test performance.* (1) Cells shall be exposed to the test substance both with and without metabolic activation. Exposure shall be for 72 hours for experiments without metabolic activation and for 48 hours for experiments with metabolic activation unless different exposure times are justified by the investigator.

(2) At the end of the exposure period, cells shall be washed and cultured to determine viability and to allow for expression of transformation.

(3) At the end of the incubation period (generally 4 to 6 weeks), cells shall be fixed and stained, and the number of transformed (Type III) foci shall be enumerated.

(4) All results shall be confirmed in an independent experiment if a single, statistically significant positive effect is produced at one dose point without a dose response. A positive response should be confirmed by testing over a narrow range of concentrations.

(5) Tumorigenic potential of isolated morphologically transformed foci may be determined by inoculation into suitable hosts.

(f) *Data and report*—(1) *Treatment of results.* (i) Data shall be presented in tabular form. Individual colony counts for the treated and control groups shall be presented for both transformation and survival.

(ii) Survival and cloning efficiencies shall be given as a percentage of the controls. Transformation shall be expressed as a number of foci per dish, the number of dishes with transformed foci, and the number of transformed foci per number of surviving cells.

(2) *Interpretation of results.* (i) There are several criteria for determining a positive result, one of which is a statistically significant concentration-related increase in the number of transformed foci. Another criterion may be based upon the detection of a reproducible and statistically significant positive response for at least one of the test substance concentrations.

(ii) A test substance which does not produce either a statistically significant concentration-related increase in the number of transformed foci or a statistically significant and reproducible positive response at any one of the test points is considered to be negative in this system.

(iii) Both biological and statistical significance should be considered together in the evaluation.

(3) *Test evaluation.* (i) Positive results for an *in vitro* mammalian cell transformation assay indicate that, under the test conditions, the test substance induces morphologic transformation in the cultured mammalian cells used.

(ii) Negative results indicate that, under the test conditions, the test substance does not induce morphologic transformation in the cultured mammalian cells used.

(4) *Test report.* In addition to the reporting recommendations as specified under 40 CFR Part 792 Subpart J, the following specific information shall be reported:

(i) Cell type used, including subclone designation and passage number; number of cell cultures; methods used for maintenance of cell cultures.

(ii) Rationale for selection of concentrations and number of cultures.

(iii) Test conditions: Composition of media, CO_2 concentration, concentration of test substance, vehicle, incubation temperature, incubation time, duration of treatment, cell density during treatment, type of metabolic activation system, positive and negative controls, length of expression period (including number of cells seeded and subculture and feeding schedules, if appropriate).

(iv) Methods used to enumerate numbers of viable cells and transformed foci.

(v) Dose-response relationship, where possible.

(g) *References.* For additional background information on this test guideline, the following references should be consulted:

(1) Heidelberger, C., Freeman, A.E., Pienta, R.J., Sivak, A., Bertram, J.S., Castro, B.C., Dunkel, V.C., Francis,

M.W., Kakunaga, T., Little, J.B., Schechtman, L.M., "Cell transformation by chemical agents—a review and analysis of the literature: a report of the U.S. Environmental Protection Agency Gene-Tox Program." *Mutation Research* 114:283–385, 1983.

(2) Kakunaga, T. "A quantitative system for assay of malignant transformation by carcinogens using a clone derived from Balb-3T3." *International Journal of Cancer* 12:463–473, 1973.

(3) Reznikoff, C.A., Bertram, J.S., Brankow, D.W., Heidelberger, C. "Quantitative and qualitative studies of chemical transformation of cloned C3H mouse embryo cells sensitive to postconfluence inhibition of cell division." *Cancer Research* 33:3239–3249, 1973.

(4) Reznikoff, C.A., Brankow, D.W., Heidelberger, C. "Establishment and characterization of a cloned line of C3H mouse embryo cells sensitive to postconfluence inhibition of division." *Cancer Research* 33:3231–3238, 1973.

(5) Sivak, A., Charest, M.C., Dudenko, L., Silveira, D.M., Simons, L., Wild, A.W. "Balb/c–3T3 cells as target cells for chemically induced neoplastic transformation." In: *Advances in modern environmental toxicology, mammalian cell transformation by chemical carcinogens*, Vol. I. Mishra, N., Dunkel, V., Mehlman, M., eds. Princeton Junction, NJ: Senate Press, pp. 133–180, 1981.

(6) Sivak, A., Tu, A.S. "Factors influencing neoplastic transformation by chemical carcinogens in Balb/c-3T3 cells." In: *The predictive value of short-term screening tests in carcinogenicity evaluation.* Williams, G.M., Kroes, R., Waaijers, H.W., Van de Poll, K.W., eds. Amsterdam, New York, Oxford: Elsevier/North Holland Biomedical Press, pp. 177–190, 1980.

(7) Williams, G.M. "Detection of chemical carcinogens by unscheduled DNA synthesis in rat liver primary cell culture." *Cancer Research* 37:1845–1851, 1977.

PART 799—[AMENDED]

2. In Part 799:

a. The authority citation continues to read as follows:

Authority: 15 U.S.C. 2603, 2611, 2625.

b. In § 799.1250 by adding paragraphs (c)(1) (ii) and (iii), (2) (ii) and (iii), (3) (ii) and (iii), (4) (ii) and (iii), (5) (ii) and (iii), and (d), to read as follows:

§ 799.1250 Cresols.

* * * * *

(c) * * *

(1) * * *

(ii) *Test standards.* (A)(*1*) *In vitro mammalian cytogenetics test.* This test shall be conducted individually with *ortho-*, *meta-*, and *para*-cresols in accordance with § 798.5375 of this chapter, except for the provisions in paragraphs (d) (3)(i) and (4) and (6) (i) and (ii).

(*2*) For the purposes of this section the following provisions also apply:

(*i*) *Type of cells used in the assay. Ortho-*, *meta-*, and *para*-cresols shall be tested in established cell lines. The cell lines or strain shall be checked for *Mycoplasma* contamination.

(*ii*) *Metabolic activation.* The metabolic activation system for this assay shall be derived from Aroclor-1254 induced rat liver S–9 preparations.

(*iii*) *Test substance—Vehicle. Ortho-*, *meta-*, and *para*-cresols shall be dissolved in DMSO prior to treatment of the cells.

(*iv*) *Exposure concentrations.* At least three concentrations of the test substance over a range adequate to define the response curve shall be tested. The highest test concentration tested with and without metabolic activation shall be 5 milligrams per milliliter or that dose which shows evidence of cytotoxicity or reduced mitotic activity.

(B) (*1*) *In vivo mammalian bone marrow cytogenetics test.* This chromosomal analysis test shall be conducted with each *ortho-*, *meta-*, or *para*-cresol isomer which produces a negative result in the *in vitro* cytogenetics test conducted pursuant to paragraph (c)(1)(i)(A) of this section. This test shall be conducted in accordance with § 798.5385 of this chapter, except for the provisions in paragraphs (d) (3)(i) and (5) (ii) and (iii).

(*2*) For the purposes of this section the following provisions also apply:

(*i*) *Animal selection—Species and strain.* The mouse shall be used. Commonly used laboratory strains should be employed. The test sponsor should provide justification/reasoning for its selection.

(*ii*) *Dose levels.* At least three dose levels shall be used. The highest dose tested shall be the maximum tolerated dose or that producing some indication of cytotoxicity, e.g., partial inhibition of mitosis, or shall be the highest dose attainable.

(*iii*) *Route of administration.* The test substance shall be administered only once by oral gavage.

(C) (*1*) *Rodent dominant-lethal assay.* This assay shall be conducted with *ortho-*, *meta-*, or *para*-cresols in accordance with § 798.5450 of this chapter, except for the provision in paragraphs (d) (3)(i) and (5)(iii) and (e)(1). The rodent dominant-lethal assay shall be conducted for each isomer which produces a positive result in either the in *vitro* or the *in vivo* cytogenetics test conducted pursuant to paragraphs (c)(1)(i) (A) and (B) of this section.

(*2*) For the purposes of this section the following provisions also apply:

(*i*) *Animal selection—Species.* The mouse shall be used. Commonly used laboratory strains should be employed. The test sponsor should provide justification/reasoning for its selection.

(*ii*) *Route of administration.* The test substance shall be administered by oral gavage.

(*iii*) *Test performance.* Each male shall be mated to no more than two, and preferably to only one, female per mating interval. Females shall be left with the males for no longer than 7 days, and mating shall continue for at least 6 weeks.

(iii) *Reporting requirements.* (A) The chromosomal aberration tests shall be completed and the final results submitted to the Agency as follows:

(*1*) The *in vitro* and *in vivo* (conditional) tests within 12 and 14 months, respectively, of the effective date of the final Phase II test rule.

(*2*) The dominant lethal assay (conditional) within 24 months of the effective date of the final Phase II test rule.

(B) Progress reports shall be submitted to the Agency for the *in vitro* and *in vivo* cytogenetics assays and the dominant lethal assay at 6-month intervals, the first of which is due within 6 months of the effective date of the final Phase II rule.

(2) * * *

(ii) *Test standards.* (A)(*1*) *Unscheduled DNA synthesis in mammalian cells in culture assay.* This assay shall be conducted with *meta*-cresol in accordance with § 798.5550 of this chapter, except for provisions in § 798.5550(d) (3)(i) and (6)(i).

(*2*) For the purposes of this section the following provisions also apply:

(*i*) *Cells—Types of cells used in the assay.* Primary cultures of rat hepatocytes shall be used.

(*ii*) *Test chemical—Vehicle. Meta*-cresol shall be dissolved in DMSO prior to treatment of cells.

(B)(*1*) *Detection of gene mutations in somatic cells in culture.* This assay shall be conducted individually with *meta-* and *para*-cresols in accordance with § 798.5300 of this chapter, except for provisions in § 798.5300(d)(3)(i), (4), (6)(i), and (e)(1).

(*2*) For the purposes of this section the following provisions also apply:

(i) *Cells—Type of cells used in the assay.* L5178Y mouse lymphoma cells shall be used. Cells shall be checked for *Mycoplasma* contamination.

(ii) *Metabolic activation.* The metabolic activation system shall be derived from the postmitochondrial fraction (S–9) of rat livers pretreated with Aroclor 1254.

(iii) *Test chemical—Vehicle. Meta-* and *para*-cresols shall be dissolved in DMSO prior to treatment of the cells. The final concentration of the vehicle shall not interfere with cell viability or growth rate.

(iv) *Test performance—Exposure.* Exposure shall be for 4 hours unless a different exposure time is justified by the investigator.

(C) *(1) Sex-linked recessive lethal test in Drosophila melanogaster.* This test shall be conducted with *meta*-cresols in accordance with § 798.5275 of this chapter, except for the provisions in § 798.5275(d)(5)(iii). This sex-linked recessive lethal test shall be conducted with *meta*-cresol if it produces a positive result in either one of the assays conducted pursuant to paragraphs (c)(2)(i) (A) and (B) of this section.

(2) For the purposes of this section the following provision also applies: *Route of administration.* The oral route of administration shall be used.

(iii) *Reporting requirements.* (A) The genetic toxicity tests shall be completed and final results submitted to the Agency as follows:

(1) The unscheduled DNA synthesis in mammalian cells in culture assay within 12 months of the effective date of the final Phase II test rule.

(2) The detection of gene mutations in somatic cells in culture assay within 12 months of the effective date of the final Phase II test rule.

(3) The sex-linked recessive lethal test in *Drosophila melanogaster*, if required, within 24 months of the effective date of the final Phase II test rule.

(B) Progress reports shall be submitted to the Agency for the unscheduled DNA synthesis in mammalian cells in culture assay, gene mutation in mammalian cells in culture assay, and the *Drosophila* sex-linked recessive lethal test at 6-month intervals, the first of which is due within 6 months of the effective date of the final Phase II rule.

(3) * * *

(ii) *Test standards.* (A) *Morphologic transformation of mammalian cells in culture.* This test shall be conducted individually with *ortho-*, *meta-*, and *para*-cresols in accordance with § 795.285 of this chapter, except for provisions in § 795.285(d)(4).

(B) For the purposes of this section the following provision also applies: *Metabolic activation. Meta-* and *para*-cresol shall initially be tested in this assay performed without metabolic activation. Only if they produce negative results in the assay performed without activation will *meta-* and *para*-cresol then be tested in the assay with metabolic activation. *Ortho*-cresol shall only be tested in this assay performed with metabolic activation.

(iii) *Reporting requirements.* (A) The morphologic transformation of mammalian cells in culture assay shall be completed and final results submitted to the Agency within 12 months of the effective date of the final Phase II test rule.

(B) Progress reports shall be submitted to the Agency for the morphologic transformation assay at 6-month intervals, the first of which is due within 6 months of the effective date of the final Phase II rule.

(4) * * *

(ii) *Test standards.* (A) *Developmental toxicity.* This study shall be conducted individually with *ortho-*, *meta-*, and *para*-cresols in accordance with § 798.4900 of this chapter. except for provisions in § 798.4900(e)(5).

(B) For the purposes of this section the following provision also applies: *Administration of test substance.* The test substance shall be administered by oral gavage.

(iii) *Reporting requirements.* (A) The developmental toxicity study shall be completed and final results submitted to the Agency within 12 months of the effective date of the final Phase II test rule.

(B) Progress reports shall be submitted to the Agency for the developmental toxicity study at 6-month intervals, the first of which is due within 6 months of the effective date of the final Phase II rule.

(5) * * *

(ii) *Test standards.* (A) *Reproduction and fertility effects.* This study shall be conducted individually with *ortho-*, *meta-*, and *para*-cresols in accordance with § 798.4700 of this chapter, except for provisions in § 798.4700(c)(5)(i)(A).

(B) For the purposes of this section the following provision also applies: *Administration of the test substance—Oral studies.* The test substance shall be administered by oral gavage.

(iii) *Reporting requirements.* (A) The reproduction and fertility effects study shall be completed and final results submitted to the Agency within 29 months of the effective date of the final Phase II test rule.

(B) Progress reports shall be submitted to the Agency for the reproduction and fertility effects study at 6-month intervals, the first of which is due within 6 months of the effective date of the final Phase II rule.

(d) *Effective date.* The effective date of the final Phase II rule for cresols is July 6, 1987.

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[FR Doc. 87–11125 Filed 5–19–87; 8:45 am]
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40 CFR Part 799

[OPTS–42030D; FRL 3202–2]

Mesityl Oxide; Final Test Standards and Reporting Requirements

AGENCY: Environmental Protection Agency (EPA).

ACTION: Final rule.

SUMMARY: On December 20, 1985, EPA issued a final Phase I test rule establishing testing requirements under section 4(a) of the Toxic Substances Control Act (TSCA) for manufacturers and processors of mesityl oxide (MO; CAS No. 141–97–7). At that time, EPA also proposed that certain TSCA health effects test guidelines be utilized as the test standards for the required studies and that test data be submitted within specified time frames. EPA has reviewed public comments on the proposal and has modified the test guidelines and time frames as appropriate. This final rule specifies these TSCA guidelines as the test standards and the reporting requirements for the testing of MO.

DATES: In accordance with 40 CFR 23.5 (50 FR 7271; February 21, 1985), this rule shall be promulgated for purposes of judicial review at 1 p.m. eastern ["daylight" or "standard" as appropriate] time on June 3, 1987. This rule shall become effective on July 6, 1987.

FOR FURTHER INFORMATION CONTACT: Edward A. Klein, Director, TSCA Assistance Office (TS–799), Office of Toxic Substances, Rm. E–543, 401 M St., SW., Washington, DC 20460 (202–554–1404).

SUPPLEMENTARY INFORMATION: On December 20, 1985 EPA issued a final Phase I rule under section 4(a) of TSCA to require testing of MO for chronic effects, mutagenicity, and oncogenicity (conditional on the mutagenicity test results). The Agency is now promulgating a final Phase II rule specifying the test standards and reporting requirements for this testing. This test rule for MO is being promulgated under 40 CFR 799.2500.